EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Glass Washing and Detergent Residues Test

SOP Number: SOP QC-03-02

Date Revised: 09-10-02

Prepared By: _____Date: ___/___/___

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Date Issued: ___/___/___

Withdrawn By: _____ Date: ___/___/___

Controlled Copy No.: _____

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1.0 SCOPE AND APPLICATION:

1.1 This protocol describes procedures for washing laboratory glassware and the method used to determine the presence of inhibitory detergent residues. Detergents used in washing glassware may leave residues which are bacteriostatic. If residues are present, glassware may require additional rinsing to remove them (see refs. 15.1 and 15.2).

2.0 DEFINITIONS: None

3.0 HEALTH AND SAFETY:

3.1 All manipulations and transfer steps involving *Staphylococcus aureus* and *Pseudomonas aeruginosa* are required to be performed in accordance with the biosafety practices stipulated in SOP MB-01, Lab Biosafety.

4.0 CAUTIONS: None

5.0 INTERFERENCES:

5.1 All glassware will be inspected prior to use. Discard items with chips and etched surfaces.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Miele Thermal Disinfector/Laboratory Glassware Washer Model G7783 serial number 16/18344823 located in room B206 (Glassware and Media Preparation Room) of the OPP Microbiology Laboratory at the ESC, Ft. Meade, MD.

7.2 Lancer1600 UP Laboratory Glassware Washer serial number 9G050714 located in room B206 (Glassware and Media Preparation Room) of the OPP Microbiology Laboratory at the ESC, Ft. Meade, MD.

7.3 Powder Detergent for Miele dishwasher

7.4 Liquid Detergent for Lancer dishwasher

7.5 Alconox Powdered Precision Cleaner

7.6 Glass and Plastic Petri Dishes (20×100 mm)

7.7 Tryptone-glucose-yeast extract-agar

7.8 Colony Counter

7.9 Twenty-four hour nutrient broth or synthetic broth cultures of test bacteria

8.0 INSTRUMENT OR METHOD CALIBRATION: Not applicable

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Detergents will be used and stored according to manufacturer's instructions.

10.0 PROCEDURE AND ANALYSIS:

10.1 Glassware will be washed in the Miele Thermal Disinfector/Laboratory Glassware Washer, Lancer1600 UP Laboratory Glassware Washer, or hand-washed.

10.2 The Detergent Residues Test will be performed annually or when a new lot or different type of detergent is used. The test bacteria used are *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

10.3 The method includes four groups of petri dishes. Preparation numbers are assigned to each group of petri dishes according to SOP QC-15, Media Prep and Sterilization Run Numbers. One group (Group A) is washed and rinsed by the regular procedure used in the laboratory (for that dishwasher or by hand). Another group (Group B) is washed by the regular procedure used in the laboratory followed by additional rinses. There will be 6 additional "Final Rinse" cycles run manually in the Miele Washer and the Lancer Washer as well as for hand-washed items. Another group (Group C) is washed with the detergent at the dilution

normally used in the laboratory but not rinsed. The last group of Petri dishes (Group D) are unwashed pre-sterilized plastic dishes tested to detect any inhibitory residues deposited during the manufacturing process. The test plates are inoculated with dilutions specified in 10.8.2 using the same test cultures as specified in 7.9.

10.4 Machine Washed Glassware in the Miele Washer

10.4.1 Glassware will be washed in the Miele Thermal Disinfector/Laboratory Glassware Washer Model G7783 using the Universal wash program (Program E). This program includes a pre-wash and heated main wash (85°C), two tap water rinses and two DI water rinses, one unheated and one heated (70°C). All rinses use recycled rinse water from the washer reservoir. This will constitute the normal treatment that all machine-washed laboratory glassware receives in this dishwasher (group A).

10.4.2 Place the glass petri dishes (24 per group) in the dishwasher spaced evenly and so the water will run out of the dish. Put half of the dishes in the lower compartment and half in the upper compartment.

10.4.3 Fill the detergent compartment with the amount suggested by the manufacturer of powder detergent and close the detergent cover. Record the amount of detergent used on the media prep sheet. Use the detergent as specified by the manufacturer. Place one scoop of detergent directly on the washer door and a second one on the base of the dishwasher for Pre-wash cycle.

10.4.4 Press the button for Program E “Universal Wash.”

10.4.5 Press the “Start” button. Allow the washer to run a complete cycle as would normally be run.

10.4.6 To achieve the additional six rinses for Group B, perform the following:

10.4.6.1 While pressing the T1 and T2 buttons, turn on the

washer.

10.4.6.2 Select program “D.”

10.4.6.3 Continuously press the start button until “38" appears in the display. Note: As the display climbs from 1 to 38, you will hear various components in the machine cycle on and off. This is normal.

10.4.6.4 When “38" is reached, press T2 until 40°C rinse temperature is reached (default value = 70°C, 40°C is the minimum temperature allowed).

10.4.6.5 Washer will automatically stop at the end of the cycle (approximately 12-15 minutes).

10.4.6.6 Repeat the above steps until the petri dishes have been rinsed six times (a total of six additional rinses).

10.4.7 To achieve Group C, the washer must be stopped after main wash cycle but before the first tap water rinse, approximately 20 minutes after starting the Universal cycle.

10.5 Machine Washed Glassware in the Lancer Dishwasher

10.5.1 Glassware will be washed in the Lancer1600 UP Laboratory Glassware Washer using Cycle 10, the designated standard laboratory wash program. This program includes a pre-wash and heated main wash (85°C), two tap water rinses and three DI water rinses, two unheated and one heated (70°C). All rinses use recycled rinse water from the washer reservoir. Liquid detergent is dispensed automatically through a metering pump at the rate of approximately 5 mL/sec in this dishwasher. The water consumption estimated per operation (according to the baskets used) is 30L. The metering pump during normal wash is set to run for approximately 75 seconds to deliver the appropriate volume of detergent. This will constitute the normal treatment that all machine-washed laboratory glassware receives (group A) in this dishwasher.

10.5.2 Place the glass petri dishes (24 per group) in the dishwasher spaced evenly and so the water will run out of the dish.

10.5.3 Select cycle 10.

10.5.4 Press the “Start” button.

10.5.5 To achieve the additional six rinses for Group B, perform the following:

10.5.5.1 Run cycle 10 for the normal laboratory wash.

10.5.5.2 Run cycle 30 twice (cycle 30 is 3 cold demineralized rinses).

10.5.6 To achieve Group C, run cycle 20 (cycle 20 is the same as the normal laboratory wash with no rinse cycles).

10.6 Hand Washed Items.

10.6.1 All hand washed items will be washed in Alconox detergent at the manufacturers' prescribed dilution (1%), rinsed with tap water, and then two times with DI water. This will constitute the normal wash treatment that all hand washed laboratory glassware receives. Wash petri dishes in this way to achieve the Group A treatment.

10.6.2 To achieve the additional six rinses for Group B, following the normal wash treatment, rinse the petri dishes six additional times with DI water (use DI water as it comes from the DI faucet not the DI water from the Barnstead Thermolyne polishing unit)

10.6.3 To achieve Group C, wash the petri dishes as in 10.6.1 omitting the tap water or DI rinses.

10.6.4 For Group D, obtain 24 pre-sterilized plastic plates and assign them a preparation number.

10.7 Sterilization of Plates.

10.7.1 Sterilize 7 plates for each Group (A, B, and C) in the usual manner (dry run for 25 min). Record the sterilization run number on the appropriate Laboratory Detergent Residue Test Form (see 16.1 and 16.2)

10.8 Test Bacteria and Pouring Plates.

10.8.1 The test bacteria used are *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

10.8.2 Target plates counts between 30 and 300 colony-forming-units are required; dilutions of 1×10^{-6}, 5×10^{-7} and 1×10^{-7} are used to achieve these levels. Refer to the Dilution Schematic for Achieving Plates with Between 30-300 Colonies per Plate (see 16.3).

10.8.3 Aliquot the appropriate dilutions into the petri plate treatments A-D in triplicate. Add 25 mL of molten 45°C TGYE agar (see SOP MB-10, Media and Reagents Used in Efficacy Testing) and swirl thoroughly to mix being careful not to spill.

10.8.4 Allow the plates to harden and incubate at 37±2°C for 24±2 hours.

10.8.5 Count colonies and record results on the appropriate form (see 16.0).

11.0 DATA ANALYSIS/CALCULATIONS:

11.1 The percent difference in colony counts between Treatment Groups will be calculated. Differences in colony counts from Group B of more than 15% indicate inhibitory residues.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the forms. Completed forms are archived in notebooks kept in locked file cabinets adjacent to offices D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule

contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The Detergent Residues Test will be performed when a new lot or different type of detergent is used; the test will be performed at least once yearly. The test bacteria used are *S. aureus* and *P. aeruginosa* (ATCC# 6538 and 15442, respectively).

13.2 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

13.3 For quality control purposes, the required information is documented on the appropriate form (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Any deviation from the protocol will be documented. If the regular wash procedure (Group A plates) is found not to be adequate for removal of inhibitory detergent residues then the wash procedure will be adjusted and the detergent residue test repeated until the wash procedure has proven that all inhibitory residues have been removed.

15.0 REFERENCES:

15.1 Bordner, R.H., J..A. Winter and P.V. Scarpino. eds. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA-600/8-78-017, Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

15.2 Eaton, A.D., Clesceri, L.S., Greenberg, A.E. eds. 1995. Standard Methods for the Examination of Water and Wastewater, 19th Edition. American Public Health Association, American Water Works Association, Water Environment Federation.

16.0 FORMS AND DATA SHEETS:

16.1 Laboratory Detergent Residue Test Form for Machine Washed Items

16.2 Laboratory Detergent Residue Test Form for Hand Washed Items

16.3 Dilution Schematic for Achieving Plates with Between 30-300 Colonies per Plate

Laboratory Detergent Residue Test Form for Machine Washed Items

OPP Microbiology Laboratory

Test Information			
Washer Name/Model		Date Test Performed	
Detergent Name		Organism	
Lot No.		Organism Control No.	
Detergent Control #			

Glassware Preparation				
Petri Dish Control #				
	Group A*	Group B*	Group C*	Group D*
Date Washed				
Date Sterilized				
Sterilization Run #				
Prep No.				

Plate Count Data								
	Date Recorded/Initials							
	Group A*		Group B*		Group C*		Group D*	
	Plate (Dil.)	CFU	Plate (Dil.)	CFU	Plate (Dil.)	CFU	Plate (Dil.)	CFU
1								
2								
3								
4								
5								
6								
7								
8								
9								
$\bar{x}$								

* The treatments/groups are defined as follows, A is normal wash, B is normal wash followed by six additional DI rinses, C is washed but not rinsed, and D is pre-sterilized plastic petri dishes.

† Differences in average counts on Groups A-D should be less than 15% if there are no toxic or inhibitory effects.

Calculations and Conclusions		
	A→B	C→B
% Difference †		

Laboratory Detergent Residue Test Form for Hand Washed Items

OPP Microbiology Laboratory

Test Information			
Washer Name/Model		Date Test Performed	
Detergent Name		Organism	
Lot No.		Organism Control No.	
Detergent Control #			

Glassware Preparation				
Petri Dish Control #				
	Group A*	Group B*	Group C*	Group D*
Date Washed				
Date Sterilized				
Sterilization Run #				
Prep No.				

Plate Count Data								
	Date Recorded/Initials							
	Group A*		Group B*		Group C*		Group D*	
	Plate (Dil.)	CFU	Plate (Dil.)	CFU	Plate (Dil.)	CFU	Plate (Dil.)	CFU
1								
2								
3								
4								
5								
6								
7								
8								
9								
$\bar{x}$								

* The treatments/groups are defined as follows, A is normal wash, B is normal wash followed by six additional DI rinses, C is washed but not rinsed, and D is pre-sterilized plastic petri dishes.

† Differences in average counts on Groups A-D should be less than 15% if there are no toxic or inhibitory effects.

Calculations and Conclusions		
	A→B	C→B
% Difference †		

OPP Microbiology Laboratory
SOP QC-03 Glass Washing and Detergent Residue Test
Dilution Schematic for Achieving Plates with Between 30-300 Colonies per Plate